COMPARATIVE ULTRASTRUCTURE OF CHLOROPLASTS IN THE SUBGENUS *EUGLENA* (EUGLENOPHYTA): TAXONOMIC SIGNIFICANCE*

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ABSTRACT — The taxonomic disposition of *Euglena geniculata* Dujardin and *E. myxocylindracea* Bold & MacEntee has been uncertain. Our electron-microscopic investigations now show that these two organisms have a chloroplast structure and morphology similar to those of other species presently classified in the Subgenus *Euglena*, *i.e.*, *E. stellata* Mainx, *E. tristella* Chu and *E. viridis* Ehrenberg. In this subgenus, chloroplasts are axial, elongate or stellate, with pyrenoids. The lobes of the stellate plastids branch repeatedly, giving rise to very narrow ribbons that extend to subpellicular regions and flank the nucleus, thus occupying much of the cytoplasm. A "paramylon center" consisting of many paramylon grains surrounds the pyrenoid and renders it invisible in the light microscope. Centrally located in the plastid, the pyrenoid matrix is penetrated by a few thylakoid pairs. Maintenance of *Euglena* strains on synthetic media in culture for many years does not cause visible changes in chloroplast morphology or ultrastructure.

RÉSUMÉ — La position taxinomique de *Euglena geniculata* Dujardin et celle de *E. myxocylindracea* Bold & MacEntee est incertaine. Nos études en microscopie électronique montrent que ces deux organismes possèdent une morphologie et une structure chloroplastiques semblables à celles d'autres espèces actuellement placées dans le sous-genre *Euglena*, *i.e.*, *E. stellata* Mainx, *E. tristella* Chu and *E. viridis* Ehrenberg. Dans ce sous-genre, les chloroplastes sont axiaux, allongés ou étoilés, et possèdent des pyrénoïdes. Les lobes des plastes étoilés sont plusieurs fois ramifiés, donnant naissance à des rubans très étroits qui s'étendent à la région sous-pelliculaire et bordent le noyau, occupant ainsi la plus grande partie du cytoplasme. Un « *paramylon center* », constitué de nombreux grains de paramylon, entoure le pyrénoïde et le rend invisible au microscope optique. Située au centre du plaste, la matrice du pyrénoïde est pénétrée par quelques paires de thylacoïdes. Le maintien de souches d'*Euglena* en culture, dans des milieux synthétiques, pendant de nombreuses années, ne provoque pas de modifications visibles dans la morphologie ou l'ultrastructure des chloroplastes. (Traduit par la Rédaction)

KEY WORDS: Chloroplast, *Euglena*, *E. geniculata*, *E. myxocylindraceu*, *E. stellata*, *E. viridis*, freshwater algae, pyrenoid, systematics, taxonomy, ultrastructure.

* We dedicate this paper to the memory of Professor P. Bourrelly. The strain of *Euglena viridis* Ehrenb. used in this investigation was isolated by him.

INTRODUCTION

The identification of intra-generic taxa of *Euglena* has been based largely on well-known morphological characters at the light-microscopic level, but especially on the historically important criterion of chloroplasts (their number, size, location; presence or absence of pyrenoids; number, morphology and location of paramylon grains). Many authorities have acknowledged chloroplast characteristics as the major criteria for the establishment of an intra-generic classification for *Euglena* (Dangeard, 1901; Lemmermann, 1913; Chu, 1946; Gojdics, 1953; Huber-Pestalozzi, 1955; Pringsheim, 1956). For most of the species of *Euglena*, however, details about the chloroplast are insufficiently known and described, a particular concern for taxa of the subgenus *Euglena* Ehrenb. (Zakryś, 1986), which is characterized by axial chloroplasts. Often obscured by other cellular structures and organelles, the plastids are not readily discernible or only faintly so in the light microscope, thus necessitating more detailed examination by transmission electron microscopy.

Of special interest for this investigation are two species tentatively classified in the Subgenus *Euglena* for which chloroplast ultrastructure is not described: *E. myxocylindracea* and *E. geniculata.* Light-microscopic observations to date on these organisms have not allowed definitive determinations about the number and morphology of the plastids, including the presence of pyrenoids. For comparative purposes two other species from the Subgenus *Euglena* (*E. stellata* and *E. viridis*) have been studied concurrently in order to document our findings. Based on earlier TEM studies it is known that these two species have single, stellate, axial plastids with pyrenoids (Dragos *et al.*, 1979). Those investigations, however, were carried out on natural populations, whereas our study is based on *Euglena* strains obtained from algal culture collections where the organisms have been maintained on synthetic media for many years. It is well known that even moderate adverse environmental conditions, such as excessive density of the population or exhaustion of nutrients, can occur in natural populations, as well as in culture and may affect chloroplast structure and morphology on a permanent basis.

The purpose of this investigation is twofold:

1. To document chloroplast number and morphology in two species of the Subgenus *Euglena* for which ultrastructural details are presently unreported (*E. myxocylindracea* and *E. geniculata*) and to compare these data to other known species of the Subgenus (*E. stellata* and *E. viridis*), also obtained from culture collections and processed for TEM with identical procedures.

2. To determine whether long-term culturing on synthetic media may result in permanent changes in chloroplast structure.

MATERIALS AND METHODS

Cultures.

All strains of *Euglena* were obtained from either the Sammlung von Algenkulturen (SAG), Göttingen, Germany, or The Culture Collection of Algae, University of Texas (UTEX), Austin, Texas, U.S.A.: *E. geniculata* Dujardin, strain #1224-4g (SAG —

isolated by E.G. Pringsheim); *E. stellata* Mainx, strain #1224-14 (SAG — isolated by F. Mainx); *E. viridis* Ehrenb., strain #1224-17b (SAG — isolated by P. Bourrelly); and *E. myxocylindracea* Bold & MacEntee, strain #1989 (UTEX — isolated by the authorities). Clones derived from each strain were cultured in liquid media (Starr, 1978) and under identical conditions in a growth chamber maintained at 19° C and 16:8 hr L/D, ca 27 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent tubes. Two-week-old cultures were used for the electron-microscopic studies.

Transmission electron microscopy.

Actively swimming cells were harvested by low-speed centrifugation (1500 g) and fixed in 2% glutaraldehyde buffered with 0,05 M sodium cacodylate, pH 7.2, for 1 hr at 4° C. After several rinses in buffer, the samples were post-fixed in buffered 2% OsO_4 also for 1 hr at 4° C, followed by dehydration in a graded ethanol/acetone series and embedment in Spurr's low-viscosity resin. Thin sections were cut with a diamond knife on a Reichert OMU-3 ultramicrotome and were stained sequentially with uranyl acetate and lead citrate, prior to examination in a Hitachi H-600 transmission electron microscope, operating at 75-100 kV.

RESULTS AND DISCUSSION

Euglena myxocylindracea. The chloroplast is a single, stellate, axial structure with a centrally located large pyrenoid (Figs 1-4). An integral part of the plastid, the pyrenoid is not surrounded by a defining membrane and is readily identifiable by its matrix, which is penetrated by a few thylakoid pairs that are continuous with the typical euglenoid triplet lamellae (Figs 5-7). The central portion of the chloroplast, containing the pyrenoid, is situated anterior to the nucleus. The pyrenoid region itself is often covered by other cellular organelles and numerous paramylon grains, termed a "paramylon center." The peripheral portions of the plastid may be divided repeatedly into narrow, ribbon-like branches that extend radially to the subpellicular regions at the cell surface, thus filling the cytoplasmic areas anterior and posterior to the nucleus (Diagram 1, Figs 1-4).

Euglena geniculata. Two axial, stellate chloroplasts of somewhat different sizes occupy the cell, the larger one anterior to the nucleus, the smaller one posterior to it. Each chloroplast contains a large central pyrenoid (Figs 19, 20). Other details of chloroplast and pyrenoid shape and structure are similar, even identical to those of *E. myxocylindracea* (Figs 21, 22, cf. Figs 1-4).

Euglena stellata and *Euglena viridis.* Similar observations of chloroplast and pyrenoid structure were made for *E. stellata* (Figs 8-13) and *E. viridis* (Figs 14-18). Long-term culture on synthetic media has not resulted in visible permanent changes in chloroplast structure. Our results on these two organisms are thus in agreement with those reported for specimens from natural populations (Dragos *et al.*, 1979).

Together with earlier investigations (Haller, 1959; Mignot, 1965, 1966; Buetow, 1968; Leedale, 1968; Dragos *et al.*, 1979; Péterfi *et al.*, 1979), our study shows convincingly that *E. myxocylindracea, E. geniculata, E. stellata, E. tristella,* and *E. viridis* have similar chloroplast structure, *i.e.*, stellate and axial, with centrally located pyrenoids (Diagram 1,

Fig 1) often obscured by a dense aggregation of paramylon grains termed a "paramylon center." The major difference among these species is chloroplast number: one per cell in *E. myxocylindracea, E. stellata,* and *E. viridis*; two in *E. geniculata*; three in *E. tristella.* Some differences in pyrenoid size between *E. stellata* and *E. viridis* were reported by Dragos *et. al.* (1979). In our opinion, however, such size differences are more likely to be due to interpopulational or even interclonal diversity, as shown for another species, *E. agilis* Carter (= *E. pisciformis* Klebs) (Zakryś & Kucharski, 1996; Zakryś *et al.*, 1996;), than to real species differences.

The hypothesis that *E. stellata* and *E. viridis* are not different species but rather clones of the same species is strongly supported by precise measurements of cell size, as well as by the great degree of DNA similarity among clones of both species (Zakryś *et al.*, in press). Nevertheless, additional research is essential for the requisite taxonomic revision of the group of species termed "*Euglena viridis* Group", Subgenus *Euglena,* in which *E. stellata* and *E. viridis*, and now *E. myxocylindracea* and *E. geniculata,* are classified (all species with morphological and chloroplast attributes similar to *E. viridis*). Such investigations will be the focus of future research.

SUMMARY

Euglena myxocylindracea has a single, stellate, axial chloroplast with a central pyrenoid, situated anterior to the nucleus, but often is not clearly discernible in the light microscope because of dense aggregations of paramylon grains termed a "paramylon center." *Euglena geniculata* has two stellate, axial chloroplasts, one anterior and one posterior to the nucleus. The centrally located pyrenoid in each plastid is frequently obscured by a paramylon center.

In both species, the pyrenoid matrix is penetrated by a few pairs of thylakoids, continuous with lamellar triplets in the chloroplast stroma. No major differences in chloroplast structure have been observed in all the species examined belonging to the Subgenus *Euglena.* Long-term culturing on synthetic media apparently has no visible, permanent effect on chloroplast structure or morphology.

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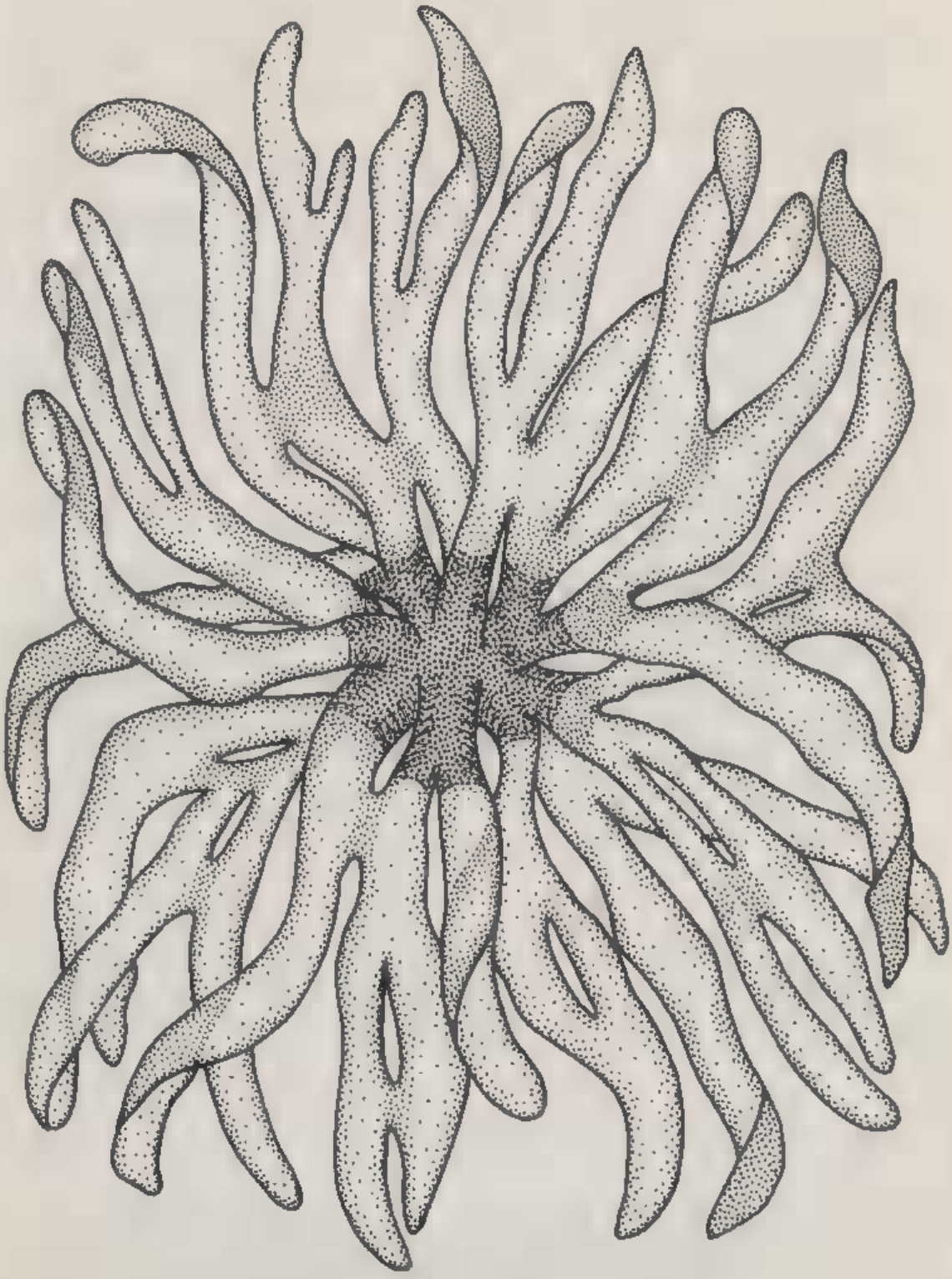


Diagram 1. Model of stellate chloroplast with centrally located pyrenoid (P).

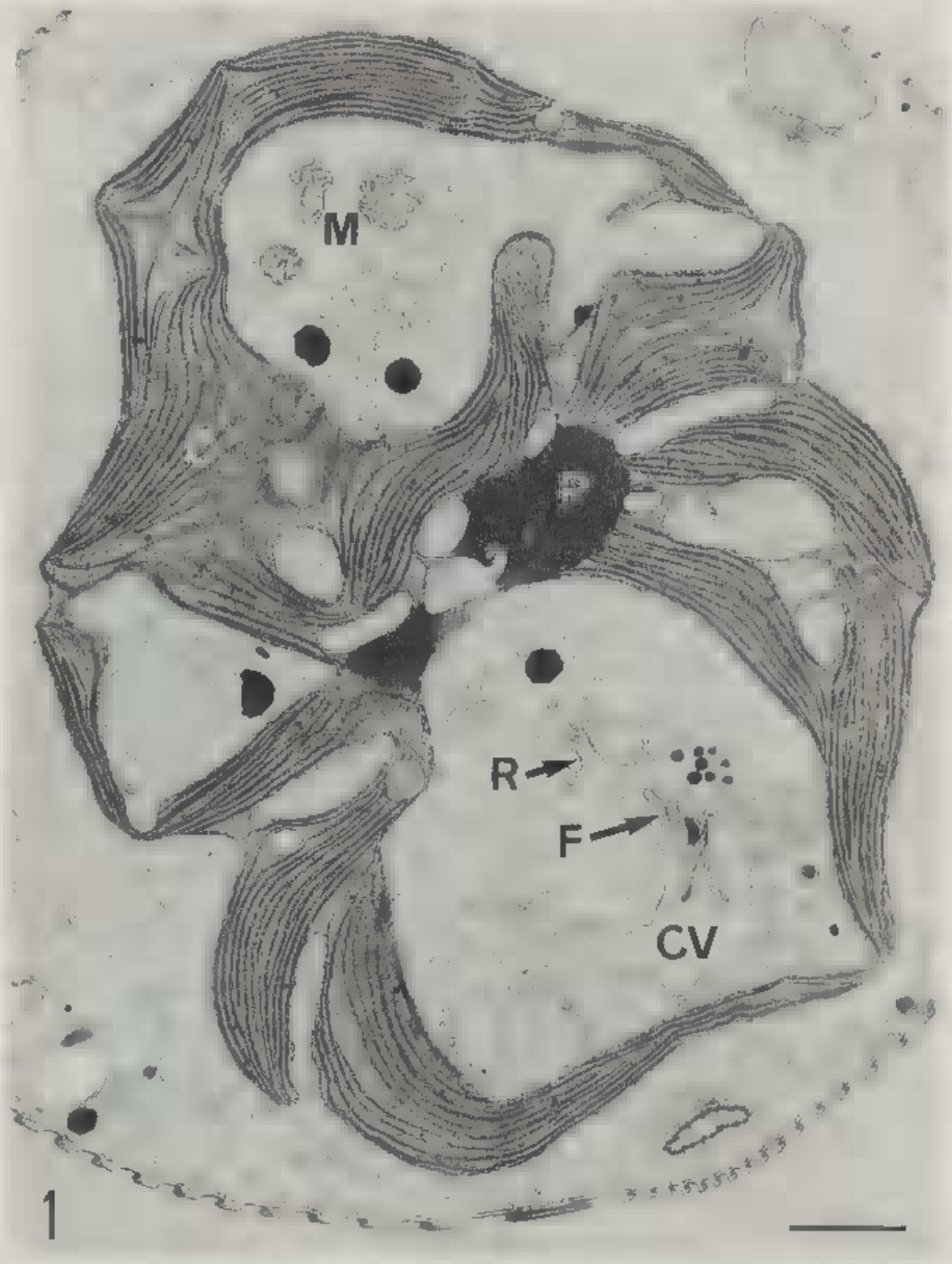
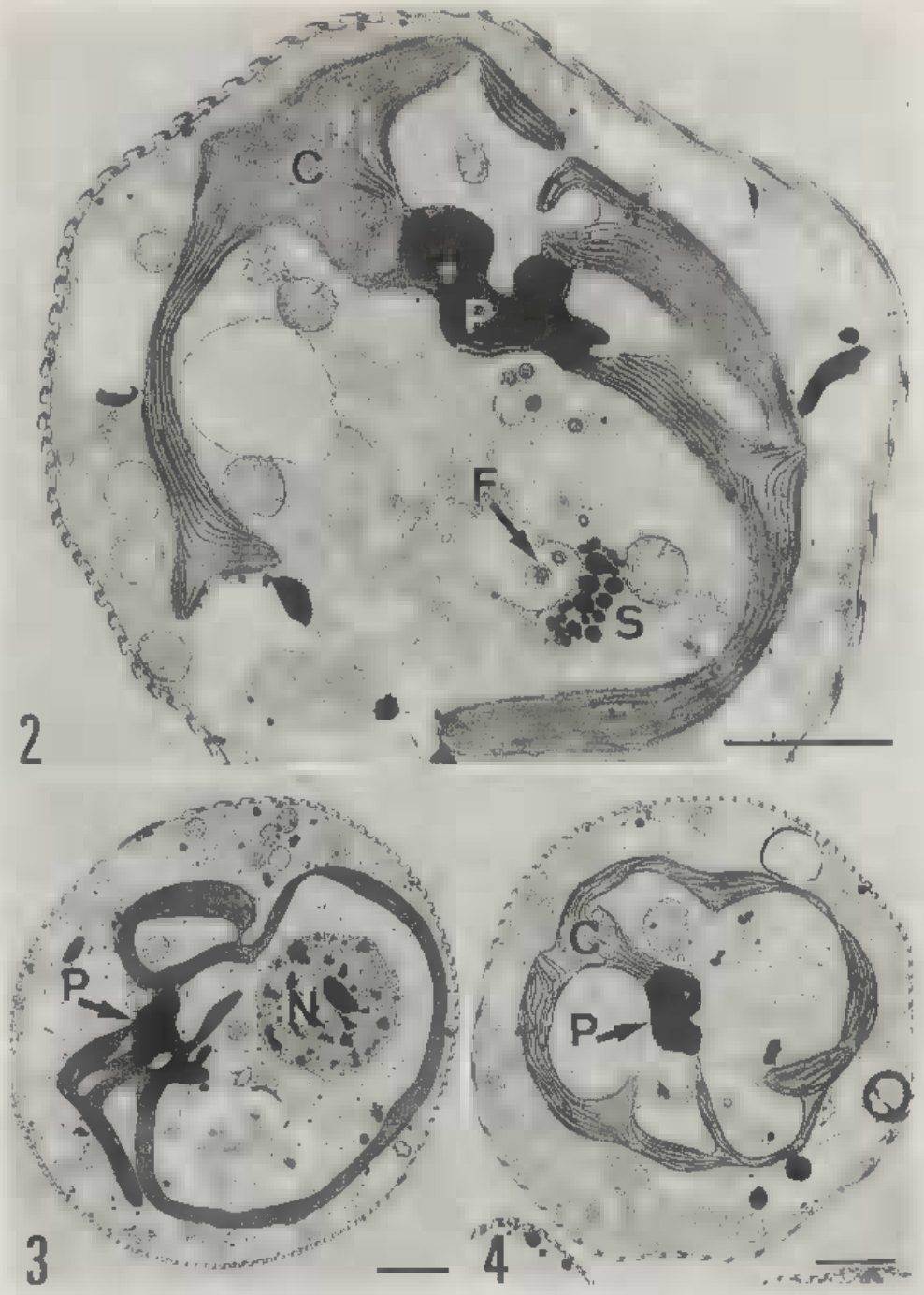
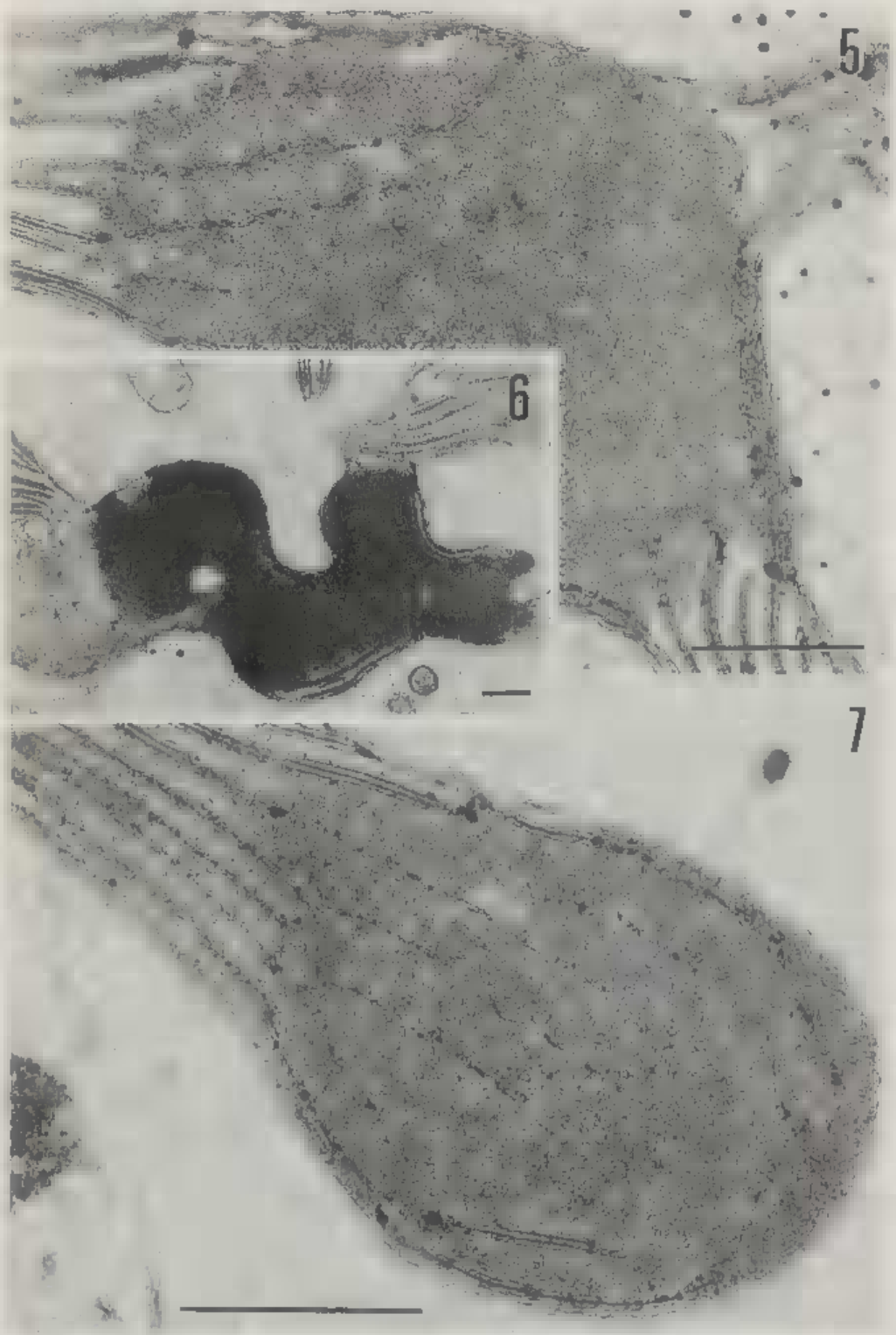


Fig. 1. *Euglena myxocylindracea.* Longitudinal oblique section shows typical cellular organization, including prominent single chloroplast (C) with pyrenoid (P), reservoir (R) with flagella (F), contractile vacuole (CV) and numerous mitochondria (M). Bar = 3 μm.

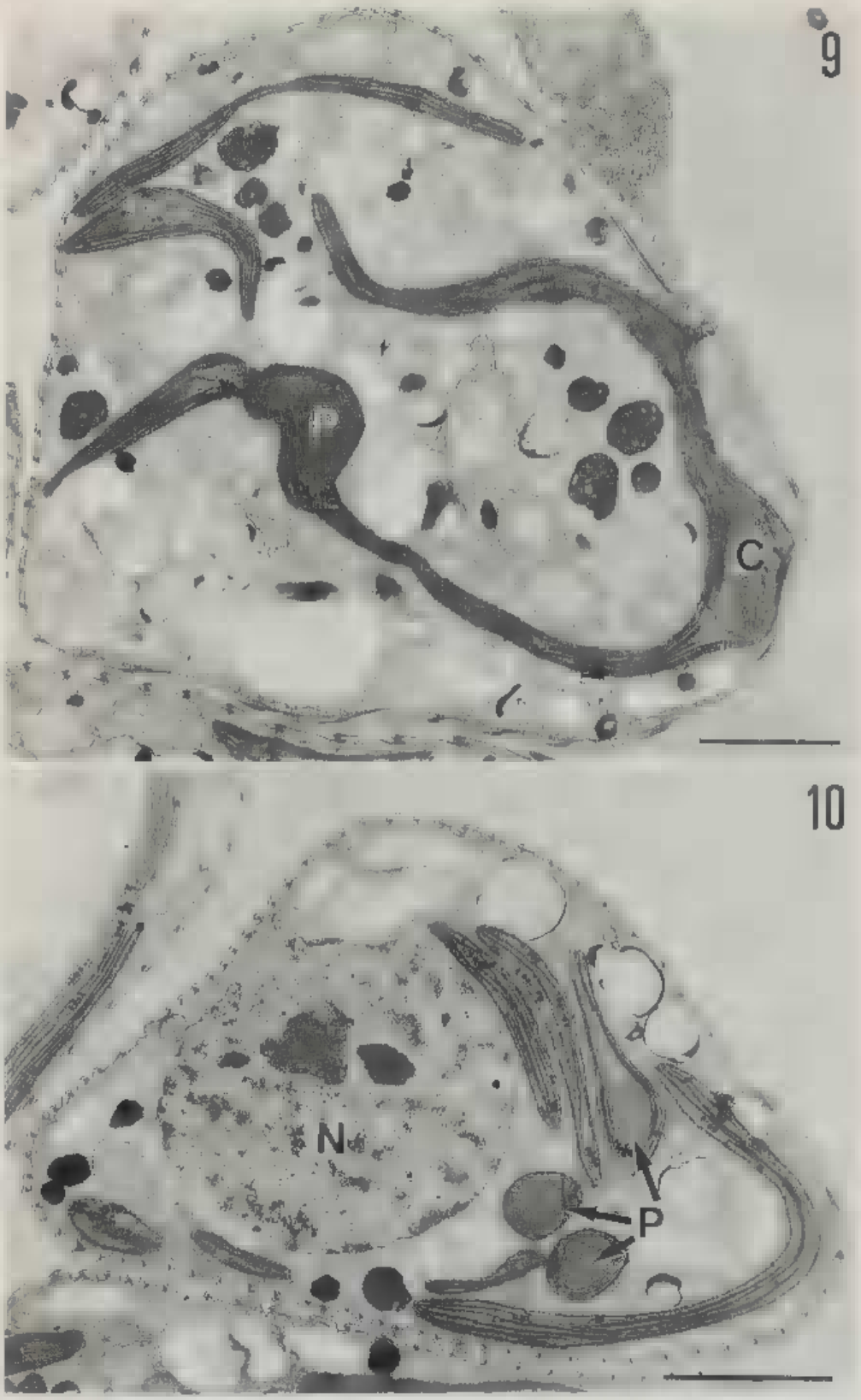


Figs 2-4. *Euglena myxocylindracea.* Transverse sections through various levels of cell show single chloroplast (C) with pyrenoid (P), nucleus (N), reservoir with flagella (F) and cytoplasmic stigma (S). Bars = 3 μm.

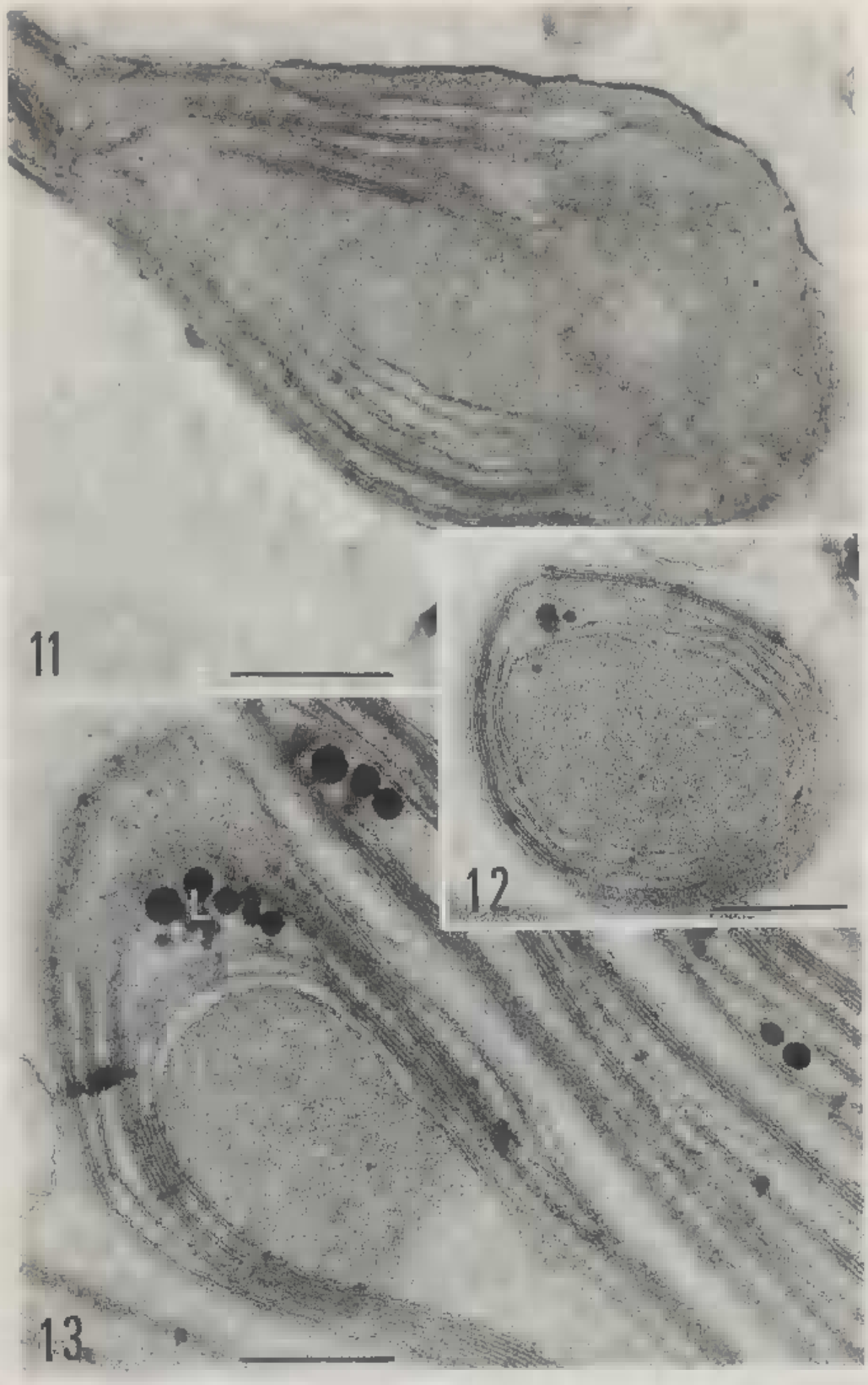


Figs 5-7. *Euglena nyxocylindracea.* Higher magnification of pyrenoids shows positional relationship of thylakoids to pyrenoid matrix. Some thylakoids extend into the matrix (cf. Figs 11,17, 21). Bars = 0,5 μm.

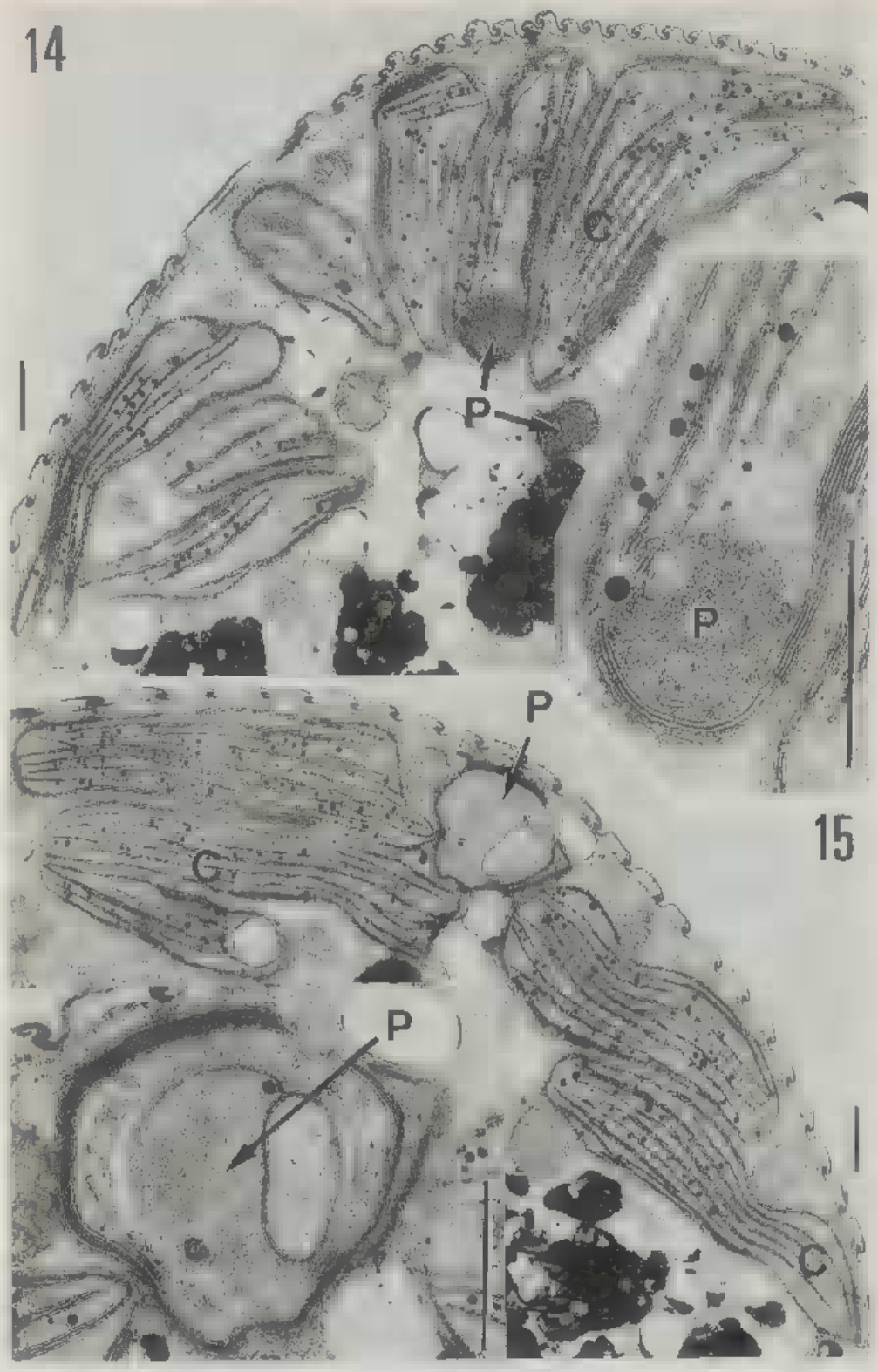
Fig. 8. *Euglena stellata*. Overview of cell in longitudinal oblique section showing chloroplast (C), nucleus (N), mitochondria (M), and numerous cytoplasmic vacuoles. Bar = 4 μm.



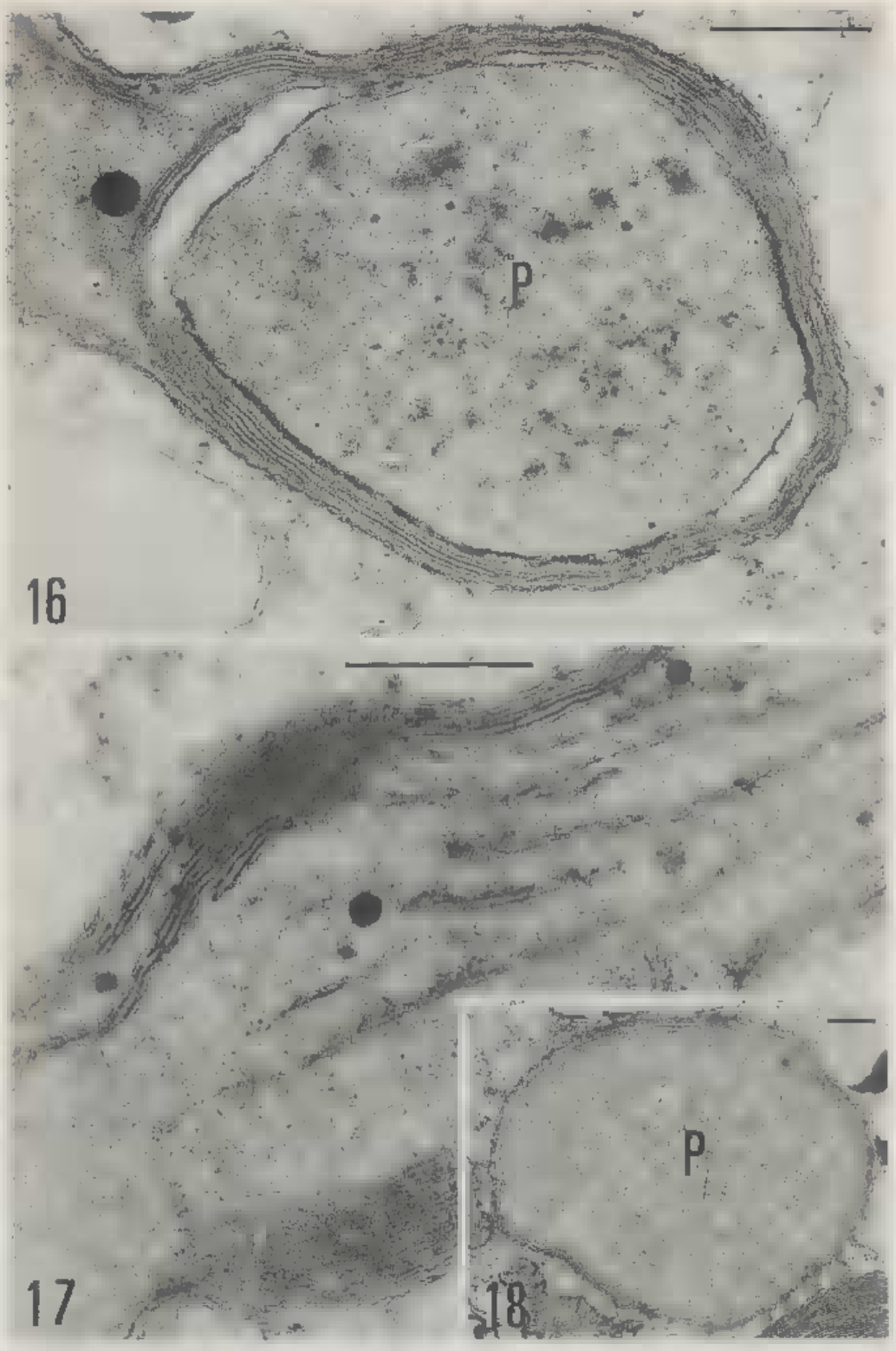
Figs 9-10. *Euglena stellata*. Transverse oblique sections at different levels showing multilobed chloroplasts (C), pyrenoids (P), nucleus (N), paramylon grains and cytoplasmic vacuoles. Bars = 3 μm.



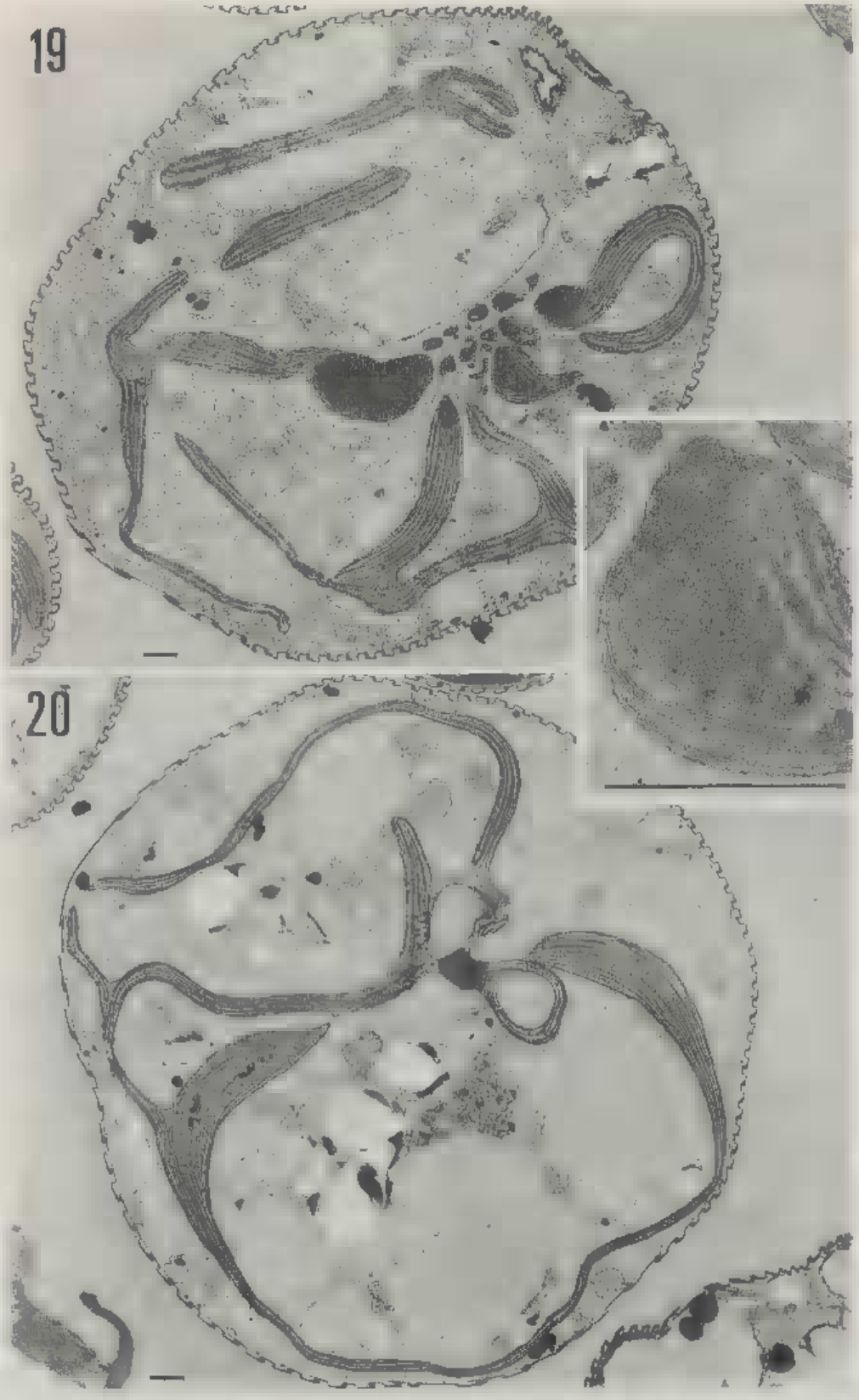
Figs 11-13. *Euglena stellata*. Higher magnification of pyrenoids shows matrix, peripheral thylakoids, and lipid granules (L). Bars = 0,5 µm.



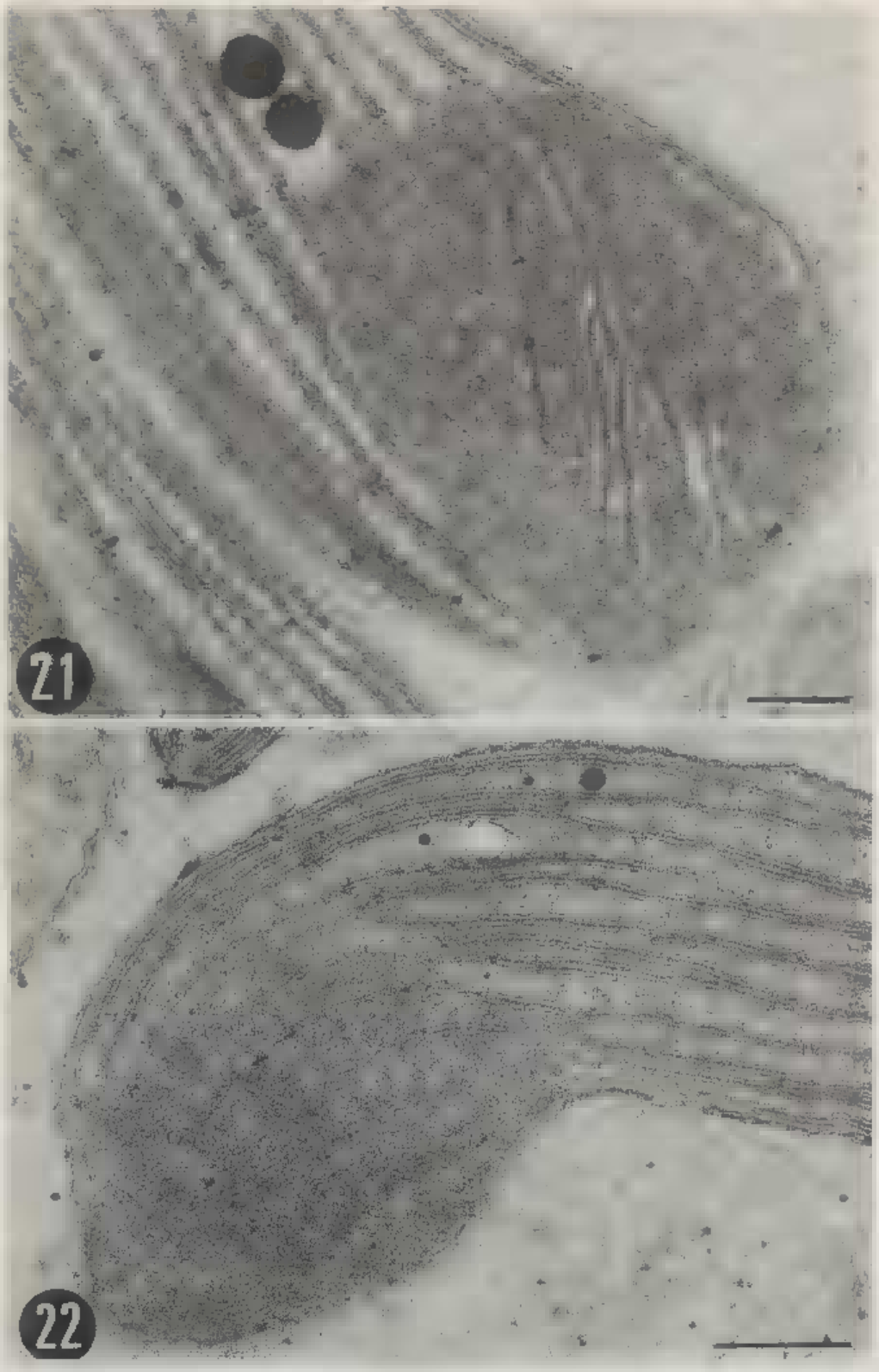
Figs 14-15. *Euglena viridis.* Portions of cells in transverse view showing peripheral chloroplasts (C) and pyrenoids (P). Insets: Higher magnification of pyrenoid regions (P). Bars = 1 μm.



Figs 16-18. *Euglena viridis.* Higher magnification of pyrenoids (P) with peripheral and traversing thylakoids. Bars = 0,5 µm.



Figs 19-20. *Euglena geniculata.* Transverse views of cells at different levels show especially the interconnected multilobed chloroplast and pyrenoid. Inset: Higher magnification of pyrenoid shown in Fig. 19. Bars = 1 μm.



Figs 21-22. *Euglena geniculata.* Higher magnification of pyrenoids showing matrices traversed by thylakoids. Bars = 0,25 µm.